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Prostaglandins in macula densa function

RAYMOND C. HARRIS, JUN-LING WANG, HUA-FANG CHENG, MING-ZHI ZHANG, and JAMES A. MCKANNA

George M. O'Brien Kidney and Urologic Diseases Center and Departments of Medicine and Cell Biology, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

Prostaglandins in macula densa function. Cyclooxygenase (COX)-2 mRNA and immunoreactive protein localize to the macula densa and adjacent cortical thick ascending limb in renal cortex, and chronic NaCl restriction increases expression of this enzyme. These findings suggest an integral role for eicosanoids generated by macula densa-associated COX-2 in mediating renin release. As selective inhibitors of COX-2 become available, it will be important to assess their effects on the renin-angiotensin system and glomerular hemodynamics.

Prostaglandins (PGs) are important physiological modulators of vascular tone and salt and water homeostasis in the mammalian kidney. In addition to their well-documented modulation of glomerular hemodynamics and distal nephron function, numerous studies suggest a role for PGs in regulation of renin production. Intrarenal arachidonic acid infusion stimulates renin release, and cyclooxygenase (COX) inhibitors suppress plasma renin [1–3]. Prostacyclin generated by the afferent arteriole mediates renin release in response to alterations in intrarenal vascular tone [4]. In addition, PGs may be mediators of macula densa (MD)-mediated renin release [5, 6]. Ito et al have found that in isolated afferent arterioles without an associated MD, renin release increases in response to prostacyclin, but not prostaglandin E_2 (PGE_2). However, with the MD attached, PGE_2 increases renin release, even in the presence of a prostacyclin synthase inhibitor [7], and more recent studies using an isolated perfused juxtaglomerular apparatus preparation have confirmed that an intact COX pathway is necessary for stimulation of renin release mediated by MD sensing of decreases in luminal NaCl [8].

The source of PGs in the MD-mediated regulation of renin secretion is still under investigation. One suggestion has been that primarily the extraglomerular mesangial cells sense altered interstitial ion concentrations. Studies in cultured mesangial cells have documented the presence of a Ca^{2+}-activated Cl^- conductance and have demonstrated that decreased ambient [Cl^-] attenuates the responsiveness of mesangial cells to vasoactive agonists such as angiotensin II and vasopressin [9] and increases PG [10] and nitric oxide production [11]. It is thus possible that the extraglomerular mesangium may respond to decreased Cl^- reabsorption by increasing PG production to stimulate renin release. Alternatively, recent studies have suggested that the thick ascending limb (TALH) and MD may be a source of PGs [12].

Prostaglandin H_2 (PGH_2) is a common precursor for all prostanoids, and its production from arachidonic acid is regulated by the enzyme PG G2/H2 synthase (COX) [13]. There are two separate gene products with COX activity: COX-1 and COX-2. Although of similar size (about 73 kDa) and with similar enzymatic activities, the isoforms share only about 66% homology in the amino acid sequence. The gene for COX-1, the constitutive isoform, encodes a 2.7–2.9 kb transcript [14], whereas the gene for COX-2, the inducible isoform, encodes a 4.2–4.5 kb transcript [15–19].

In the kidney, immunoreactive COX-1 is found in highest concentrations in mesangial cells, arteriolar endothelial cells, parietal epithelial cells of Bowman's capsule, and the collecting duct and medullary interstitial cells. No immunoreactive COX-1 is found in Henle's loop or MD [20].

COX-2 mRNA was first detected in phorbol ester-treated Swiss 3T3 cells, and its expression has been shown subsequently to increase in a variety of cultured epithelial, mesenchymal, and endothelial cells in response to serum, growth factors, and inflammatory stimuli [16–19]. COX-2 is the steroid-sensitive isoform of COX, because glucocorticoids decrease COX-2 expression by both transcriptional and posttranscriptional mechanisms [17, 21]. COX-2 expression in the kidney increases after systemic administration of lipopolysaccharide [22]. We have also shown localized expression in the developing and adult kidney that is regulated by noninflammatory stimuli. Using a cDNA probe specific for COX-2, we determined constitutive expression of COX-2 mRNA in normal rat kidney. Immunoblotting of microsomes from rat kidney cortex and papilla with an antiserum specific for COX-2 revealed immunoreactive protein with a molecular weight of about

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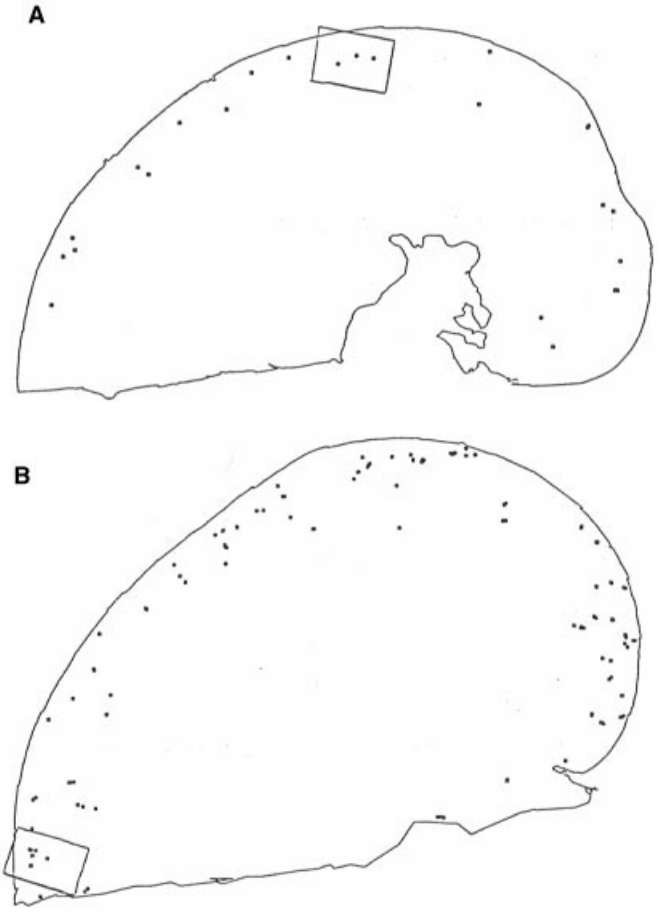

Fig. 1. Topographic distribution of cyclooxygenase (COX)-2 immunoreactive protein in the kidney. Representative renal sections from control (*A*) and low-salt (*B*) rats. The section profiles and locations of COX-2 positive cells were determined at a resolution of [plusminus] 1 μm (reprinted from [12] with permission).

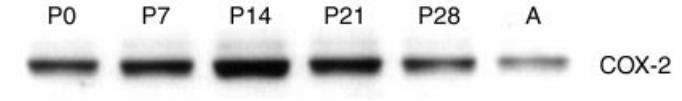

Fig. 2. Cyclooxygenase (COX)-2 expression in kidney homogenates of rats staged from newborn (P0) to adult. Equivalent amounts of protein loaded in each lane were separated by gel electrophoresis and transferred to a membrane for immunochemical identification with COX-2 antibody (reprinted from [39] with permission **from the Journal of Clinical Investigation**).

73 kDa in both locations, with greater relative abundance in papilla. *In situ* hybridization revealed a restricted distribution of COX-2 mRNA. In the cortex, it was localized to cells of the cortical TALH (cTALH) in the region of the MD. Immunohistochemical studies confirmed that a restricted subset of cells in the outercortex and midcortex expressed COX-2. These cells were identified as the epithelial cells of the MD and the adjacent cTALH. The immunoreactivity of stained cells was intense, but only one (and rarely two) COX-2—positive cell was observed per site. The majority of identified glomeruli sectioned through the juxtaglomerular apparatus had no COX-2—positive cells in the MD. In the papilla, medullary interstitial cells were COX-2 positive, with the greatest incidence of immunoreactivity at the papillary tip. No COX-2 immunoreactivity was detected in arterioles, glomeruli, or cortical or medullary collecting ducts [12].

To determine whether alterations in volume status alter cortical COX-2 expression, additional rats were volume expanded by administering 1% NaCl in drinking water or volume contracted by placing them on a low-sodium diet. The number of cells displaying immunoreactive COX-2 in the cortex did decrease in the volume-expanded animals, but the baseline was so low that statistical significance was not reached. In contrast, chronic dietary salt depletion increased cortical COX-2 immunoreactivity significantly from 0.86 ± 0.08 ($N = 3$) to 2.52 ± 0.43 ($N = 4$) cell clusters of COX-2 immunoreactivity/mm^2 cortex ($P < 0.025$). Similarly, the percentage of glomeruli with an associated COX-2—immunoreactive MD increased from 5 ± 1.2 to $16.1 \pm 3.3\%$ ($P < 0.05$). In addition to increased frequency of sites with COX-2—positive cells, more cells of each MD and associated TALH were positive in the cortices of low-salt animals. The number of individual COX-2 immunoreactive cells increased from 0.95 to 4.99 cells/mm^2 cortex, and the total area of the COX-2 immunoreactive cells in cortex increased from 34 to 226 μm^2/mm^2.

PGE_2 inhibits net Cl^- reabsorption in the (non-MD) cells of the TALH [23]. Locally produced PGs may thus function as autacoids to inhibit MD transport directly. Alternatively, PGs may affect signaling by the extracellular mesangial (Goormaghtigh) cells [11]. Finally, although epithelial cells of more distal nephron structures produce predominantly PGE_2 [24], the profile of COX products produced by MD cells is unknown. Although *in vitro* studies have not indicated differences between the profile of PGs produced by purified or recombinant COX-1 and COX-2, it is possible that MD cells may produce PGI_2 and/or other COX metabolites that directly stimulate renin secretion by JG cells. Although the metabolites involved or signaling have still not been determined, a recent study by Harding et al has demonstrated that administration of a selective COX-2 inhibitor prevents increases in renal renin mRNA levels in response to imposition of a low-sodium diet [25].

The mechanisms by which renal cortical COX-2 are regulated are still under investigation. Given that glucocorticoids inhibit COX-2 expression in inflammatory states, it is of interest that we have found significant increases in cTALH COX-2 expression in rats after adrenalectomy [26]. In preliminary studies, we have found also that renal cortical COX-2 expression in rats on low-salt diets is increased significantly with concomitant treatment with an angiotensin converting enzyme inhibitor or angiotensin I

receptor antagonist [27], suggesting negative feedback by angiotensin II.

It is of interest that COX-2, but not COX-1, is found in the MD. One possible explanation for this selectivity is that rapid and transient transcriptional regulation of COX-2 would allow the MD to modulate its PG production to provide appropriate regulation of the renin/angiotensin system. The localization of COX-2 to the MD also raises the possibility that alterations in COX-2 activity may be involved in renin-dependent hypertension. In this regard, in a model of renovascular hypertension in rats [28], indomethacin decreased systemic blood pressure. Similarly, in humans with renovascular, but not essential, hypertension, i.v. aspirin significantly reduced systemic blood pressure [29].

Evidence suggests that COX metabolites may play important functional and developmental roles in the fetal kidney. Mateson et al have found that administration of the nonspecific COX inhibitor indomethacin to fetal lambs during the third trimester of gestation increased renal vascular resistance, decreased fetal renal blood flow, increased urinary sodium and chloride excretion, and decreased plasma renin activity, indicating that COX metabolites are important for maintenance of fetal renal function [30]. The incidence of oligohydramnios is reportedly increased in women who chronically consume significant amounts of aspirin or other COX inhibitors during the third trimester of pregnancy [31]. Because the fetus is the source of a significant amount of amniotic fluid, these studies further suggest an important role for COX metabolites in maintenance of fetal renal function.

There is also evidence for a role of COX metabolites in mediation of normal renal development. Chronic administration of indomethacin to pregnant Rhesus monkeys leads to renal hypoplasia, with kidney size decreased to 15% of control animals. The observed defect was specific for the kidney, because in the treated animals, development of the other organs was not affected, except that the livers were larger in the treated infants [32]. Human fetal renal maldevelopment has also been reported with chronic use of COX inhibitors during pregnancy. The kidneys from these infants who came to term or died in the early postnatal period were noted to have few differentiated proximal tubules in the inner cortex, associated with crowding of the glomeruli. The outer cortex was more severely affected, with evidence of poorly differentiated glomeruli, undifferentiated tubule epithelia, and tubular dilation. In addition, the medullary pyramids were crowded with small immature tubules [33, 34]. Further evidence for a potential role of COX metabolites in nephrogenesis has been provided by studies of mouse metanephric cultures. Avner et al, studying growth requirements of embryonic (E) day 13 mouse metanephric explants grown in the absence of fetal calf serum, have determined that PGE_1 is necessary for maximal growth and differentiation [35]. In addition to an approximately 33% decrease in overall cellularity of the metanephric kidney grown in the absence of PGE_1, addition of PGE_1 to the basal media was an essential prerequisite for induction of metanephric differentiation (measured as development of epithelial glomeruli formation), and PGE_1 by itself was almost as effective in promoting differentiation as a complete hormonally defined medium (selenium, insulin, transferrin, thyroxine, and PGE_1) [35].

Two recent reports of targeted disruption of murine COX-2 have also indicated an important developmental role for this enzyme in renal development [36, 37]. The kidneys of homozygous (COX-2−/−) animals are small, with a paucity of functional nephrons, undeveloped mesenchymal tissue, and immature glomeruli and dysplastic tubules in the outer cortex. Microcystic lesions are found in the corticomedullary junction and are accompanied by hypoplasia or atrophy of the medulla. The average life span of these mice is 3.5 months, and they die of uremia. The pathologic presentation appears to be very similar to the previous descriptions of renal abnormalities in infants of mothers consuming large quantities of nonsteroidal anti-inflammatory drugs. No apparent developmental or functional abnormalities have been described in mice with targeted disruption of COX-1 [38].

We have determined that in normal rat kidney development, COX-2 mRNA and immunoreactive protein are present in the metanephric kidney from day E16 and COX-2 is apparent in cells of both the collecting ducts derived from ureteric buds and from the s-shaped bodies [39]. In late gestation, localized cells with intense COX-2 immunoreactivity were noted in the developing cTALH. This expression peaked in the third postnatal week and declined to adult levels by the third postnatal month (Fig. 2). Immunolocalization and *in situ* hybridization demonstrated intense COX-2 expression in a subset of cTALH near the MD in each developing nephron.

The placenta is a rich source of PGs, and in ovine placenta, COX-2 expression increases in the second half of pregnancy [40]. Therefore, if PGs or other COX metabolites play an important role in renal development, it is possible that maternally produced products may be a contributory source during pregnancy. In the mice with targeted disruption of COX-2, heterozygotes have apparently normal COX-2 expression and function, indicating that the observed renal abnormalities are due to the absence of fetal COX-2. However, in these studies, it is apparent that postnatal nephrogenesis also is severely disrupted, as evidenced by the severe lesions noted in outer cortical nephrons. Nephrogenesis occurs centrifugically, and in the rodent, postnatal nephrogenesis continues for the first 14 to 20 postnatal days. Therefore, the finding that postnatal cortical COX-2 expression is highest in the first few weeks of postnatal rats is consistent with a continuing role in postnatal nephrogenesis.

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Reprint requests to R.C. Harris, M.D., Division of Nephrology, Department of Medicine, S-3223 MCN, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, USA.
E-mail: Ray.Harris@mcmail.vanderbilt.edu

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